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Research Article

Chemical composition and potent antibacterial activities of colony-forming cyanobacteria, *Desmonostoc muscorum* (Nostocales, Cyanophyceae)

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Abstract: Cyanobacteria are important natural sources of biomolecules and active compounds with promising biological activities against a wide range of microbial pathogens. The study aimed to evaluate the chemical composition and antibacterial activities of colony-forming cyanobacteria, *Desmonostoc muscorum*. Proximate analysis showed that *D. muscorum* biomass possesses high concentration of carbohydrates (35.50 ± 0.12%), protein (20.19 ± 0.03%), and ash (16.90 ± 0.02%). The elemental composition of *D. muscorum* biomass is in a decreasing order of Ca > Mn > Mg > K > Na > Fe> Zn > Cr > Pb > Cu > Cd. Also, *D. muscorum* extract exhibited potent antibacterial activities against *Staphylococcus saprophyticus*, Methicillin-Resistant *Staphylococcus aureus*, and *Listeria monocytogenes* with MIC values of 125 µg/mL, 125 µg/mL, and 250 µg/mL, respectively. The current study documents the promising use of *D. muscorum* as good sources of microelements and compounds which can be harness for food and medical applications.

1. INTRODUCTION

An increasing number of reported cases of disease (caused by drug-resistant microorganisms) causes a significant risk to public health. Among microbial pathogens, viruses and bacteria cause 37– 70% of diseases while protozoa, fungi and helminths cause 10–30% of diseases, resulting in several deaths per year (Hirata *et al.*, 1996; Ganesan *et al.*, 2017). Recently, bacterial infections are becoming resistant to several antibiotics causing a foremost worldwide healthcare problem. For instance, *Staphylococcus aureus*, a pathogenic bacterial strain responsible for several human infections, has gained resistance to majority of clinically important antibiotics. Clinicians reported cases of hospital acquired (nosocomial) drug resistant strains of *S. aureus*, which also contains resistance to a wide array of antibiotics (Kumar *et al.*, 2010). Thus, there is a high demand to continuously discover novel bioactive compounds. Regardless of all the efforts to synthesize chemically active compounds, natural environmental samples are still the best source of new compounds (Hirata *et al.*, 1996 Kumar *et al.*, 2010; Salehghamari & Najafi, 2016; Żymańczyk-Duda *et. al.*, 2022).

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Microorganisms are considered one of the significant sources of bioactive compounds with strong antimicrobial activity over a wide array of bacterial pathogens. In general, microorganisms produce biologically active molecules that are non-essential for their growth but beneficial in defense mechanism (Ganesan *et al.*, 2017; Senhorinho *et al.*, 2018; Żymańczyk-Duda *et. al.*, 2022). Cyanobacteria are source of several active substances that have diverse applications and clinical effects in human medicine. In fact, several naturally derived antibiotics were extracted from this group of bacteria. In addition, cyanobacteria are efficient photosynthetic microorganisms that collect sunlight and convert it to useful biomass (majority of which is carbohydrate, protein, and lipids) which can be harnessed for food and industrial application (Tibbets *et al.*, 2015 Little *et al.*, 2021; Żymańczyk-Duda *et. al.*, 2022). These organisms are found in several ecological habitats such as water, soil, lake, and other marine environment. Different types of cyanobacteria are recognized for generating both internal and external compounds that possess a wide range of biological activities against bacteria, fungi, and other viruses. The production of these antimicrobial agents is influenced significantly by factors such as the temperature and pH during incubation, the duration of incubation, the components of the medium, and the intensity of light (Orhan *et al.*, 2003; Katircioglu *et al.*, 2005). Although cyanobacteria have been the subject of extensive research for bioactive compound screening by medical companies for almost 50-60 years, only a minimal portion of the cyanobacterial taxa has been reported (Orhan *et al.*, 2003; Shaieb *et al.*, 2014 Salehghamari & Najafi, 2016; Senhorinho *et al.*, 2018). Cyanobacteria such as *Microcoleous lacustris* are being used as good source of abietane diterpenes with potent antibacterial activities against *S. aureus, S. epidermidis, Salmonella typhi, Vibrio cholerae, Bacillus cereus, Bacillus subtilis, Escherichia coli,* and *Klebsiella pneumonia*. (Cock & Cheesman, 2023). In addition, *Fischerella ambigua*, *Anabaena basta*, *Nostoc commune*, and *Spirulina platensis* produce medically important compounds such as ambiguine isonitriles, bastadin, comnostins A-E, and calcium spirulan which are being used to treat infection caused by *Bacillus anthracis* and *Mycobacterium tuberculosis* (Katircioglu *et al.*, 2005; Cock & Cheesman, 2023). Despite these advancements, further investigations are essential to identify and evaluate the therapeutic potential of several bioactive compounds derived from cyanobacteria. Antimicrobial substances from cyanobacteria need more research that will target the safety of these compounds in antimicrobial chemotherapy, given that certain cyanobacterial species produce environmental toxins (Orhan *et al.*, 2003; Little *et al.*, 2021; Cock & Cheesman, 2023). Nevertheless, many of these compounds display relatively low toxicity, making them promising candidates for drug development. Notably, some heterocyclic and linear peptides, as well as depsipeptides, have exhibited potent activity and favorable safety profiles, prompting their development as antimicrobial chemotherapies (Katircioglu *et al.*, 2005; Żymańczyk-Duda *et. al.*, 2022; Cock & Cheesman, 2023).

Desmonostoc muscorum is a colony-forming, filamentous nitrogen-fixing cyanobacterium capable of exhibiting wide array of physiological growth properties and cell life cycle (developmental stages) alternatives. This cyanobacterium is currently being studied for medical application due to its potent antibacterial and antioxidant properties (El-Sheekh, *et al.*, 2006; Yasin *et al.*, 2019). However, studies on the biological activities of this cyanobacterium are still limited, particularly the chemical and elemental composition as well as the antimicrobial properties of the cyanobacteria. Thus, the study aimed to evaluate the chemical composition and antibacterial activities of *Desmonostoc muscorum* BIOTECH 4087 isolated from rice paddies in Laguna, Philippines.

2. MATERIAL and METHODS

2.1. Microalgal Culture and Mass Production

The pure culture of cyanobacterium, *Desmonostoc muscorum* BIOTECH 4087 was obtained from the Philippine National Collection of Microorganisms (PNCM), National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños

(UPLB) (Figure 1). Briefly, 100 mL of *Desmonostoc muscorum* culture was grown into three 1 L flasks containing BG 11 medium (Arguelles, 2022; Arguelles, 2023). The culture media used in this study was sterilized using autoclave at 15 psi for 15 min. Mass production of *D. muscorum* was done for 24 days under 12:12 light condition (light intensity = 120 µmol photons m^{-2} s^{-1}) and kept at 23 ± 2 °C. The culture set up was bubbled continuously with filtered air from an air pump (gas velocity = 300 mL min^{-1}). The biomass of *D. muscorum* was collected using centrifugation (10,000 rpm for 10 min). The collected biomass was freeze-dried via Virtis Freeze mobile 25 SL lyophilizer to generate dried cyanobacterial biomass (Arguelles, 2023).

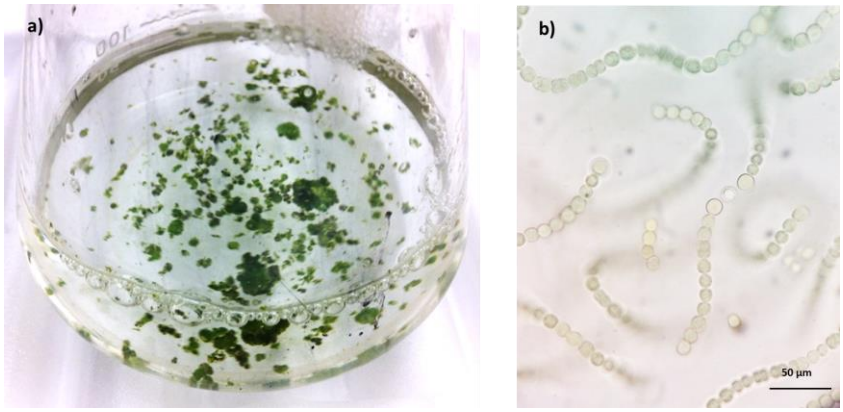

Figure 1. *Desmonostoc muscorum* BIOTECH 4087 in cultured flask (a) and a photomicrograph of the heterocystous filaments (b).

2.2. Cyanobacterial Extract Preparation

The algal extract was prepared by subjecting 1 gram of freezed-dried biomass of *D. muscorum* in 20 mL of methanol placed in an ultrasonic bath with continuous stirring for 1 hr. The extraction mixtures were centrifuged for 10 min at 12,000 rpm with a temperature set at 4 °C. Following extraction, the crude algal extracts underwent a decolorization process employing activated charcoal bleaching to remove pigments in the extract. Briefly, 1 mg/mL of activated charcoal powder was applied to the crude extract. Following the addition of activated charcoal and vortexing for 1 minute, the mixture underwent centrifugation for 5 minutes at 2,000 g, and the resulting supernatant was filtered through a polytetrafluoroethylene (PTFE) (0.22 µm) membrane. The cyanobacterial extracts were further concentrated via a rotary evaporator and were kept at 4°C (refrigerated condition) before use in the different antibacterial assays (Tzima *et al.*, 2020; Arguelles, 2022; Arguelles, 2023).

2.3. Proximate Analysis

2.3.1. *Moisture content*

Approximately 5 grams of *D. muscorum* biomass were measured into an evaporating dish. The cyanobacteria were then placed in an oven, maintained at 105 °C for a minimum of 5 hours. Subsequently, the sample was transferred to a desiccator to cool at room temperature before being weighed. The sample was subjected to heating in the oven for another 30 minutes, followed by cooling and reweighing. This process was repeated until successive weighings did not deviate by more than 0.001 gram (Arguelles et al., 2019; Arguelles, 2023).

2.3.2. *Fat content (Soxhlet method)*

Approximately 1 gram of *D. muscorum* biomass was measured into a filter paper thimble and subjected to drying in an oven for a duration of 2 hours. The treated biomass of *D. muscorum* was then transferred into an extractor, utilizing a pre-weighed Soxhlet flask, and extracted with ether for 16 hours. After extraction, the sample was extracted from the solvent, and the solvent was recovered. The Soxhlet flask containing the lipid was subsequently dried on a hot plate for 5 minutes, or until the solvent had evaporated, then allowed to cool before being weighed (Arguelles, 2023). Crude fat was calculated as follows:

$$Lipid\ Content\ (\%) = \left(\frac{Weight_{lipid}}{Weight_{sample}}\right) \times\ 100$$

2.3.3. *Ash content*

The biomass of *D. muscorum* underwent ignition in a muffled furnace at 550 ºC for 2 hours. Subsequently, it was allowed to cool to 50ºC before being transferred to a desiccator. After reaching room temperature, the sample was weighed, and then subjected to repeated ignition at 30-minute intervals until no further weight loss was observed. The calculation of ash content proceeded as follows:

$$Ash\ Content\ (\%) = \left(\frac{Weight_{ash}}{Weight_{sample}}\right) \times\ 100$$

2.3.4. *Crude protein (Kjeldahl) method*

Protein content was determined using Kjeldahl method, which involves analyzing the total nitrogen present and then multiplying this value by a specific factor appropriate for the sample type. The protein content was expressed as grams per 100 grams of the edible portion (Arguelles, 2023).

2.3.5. *Crude fiber content (Weende method)*

Approximately 0.3 grams of fat-free *D. muscorum* biomass were weighed out in a 500 mL Erlenmeyer flask. To this, 200 mL of H_2SO_4 under boiling conditions was added. The flask, attached to a condenser, was heated for 30 minutes, with regular rotation to ensure thorough contact of samples with the solution. After this duration, the mixture was promptly filtered through a linen cloth in a stemless funnel and washed immediately with hot distilled water until the washings were no longer acidic. The residue remaining on the cloth was rinsed back into the Erlenmeyer flask using 200 mL of boiling NaOH. The flask, again attached to a condenser, was boiled for another 30 min. The residue was then filtered once more through cloth in a funnel, washed with sterile distilled water, and quantitatively transferred back to the flask. The residue was filtered through a gooch crucible lined with a thin layer of asbestos. The contents and crucible were dried at 110ºC to achieve constant weight and ignited at 600ºC until carbonaceous matter has been consumed. The weight loss incurred during this process was recorded as crude fiber (Arguelles *et al.*, 2019; Arguelles, 2023).

2.3.6. *Carbohydrate content*

Carbohydrate concentration was calculated by subtracting the total of crude fat, ash, moisture, crude fiber, and crude protein from 100. Zero value was assigned to carbohydrates if the sum of fat, water, protein, and ash is more than 100 (Arguelles, 2023).

$$\%Carbohydrate = 100 - (\%Moisture\ Content + \%Protein + \%Fat + \%Ash)$$

2.4. Elemental Composition Analysis

Desmonostoc muscorum biomass was treated to dry ashing to determine the elemental composition following the standard methods (Arguelles, 2023). Initially, 1 g of *D. muscorum* biomass was dried using a muffle furnace for 5 h set at 550 °C. The drying of the algal biomass was repeated until a whitish or gray residue was obtained. The remaining residue was further dissolved in 10 mL HCl and by heating slowly the reaction mixture. The solution was shortly put to a hot plate (temperature set at 100 °C) for the remaining ash to dissolve. The collected solution was then filtered using a filter paper (Whatman) and kept in a small flask. Detection and quantification of magnesium, iron, cadmium, sodium, calcium, lead, manganese, potassium, zinc, chromium, and copper were done using an atomic absorption spectrophotometer Perkin Elmer AAnalyst 400 (Arguelles, 2022; Arguelles, 2023).

2.5. Disk Diffusion Assay

Three Gram-positive bacteria (*Listeria monocytogenes* BIOTECH 1958, Methicillin-Resistant *Staphylococcus aureus* BIOTECH 10378, and *Staphylococcus saprophyticus* BIOTECH 1802) and one Gram-negative bacteria (*Pseudomonas aeruginosa* BIOTECH 1824) were tested against *D. muscorum* crude extract using paper disc assay (Elfita *et al.*, 2019; Arguelles, 2022). Bacterial test pathogens were initially cultured in Luria Bertani broth medium and kept for 24 hours at 35 ℃ under shaking condition. Briefly, 25 mL of Mueller-Hilton agar (MHA) was poured in petri plates. A sterile swab was dipped into the inoculum tube (cell density was adjusted to equal turbidity of 0.5 McFarlands) and inoculated into the surface of dried MH agar plate. The swab was streaked three times in the entire surface of MHA agar. Three paper discs were placed on the agar surface. One paper disc was dipped in *D. muscorum* extract while the other two paper were dipped in 1000 ppm tetracycline (positive control) and methanol (negative control). Each plate was kept for 18 hours at 35°C. Inhibition zones were measured for the control antibiotic as well as *D. muscorum* extract and were expressed as percentage activity. Biocidal activity of *D. muscorum* extract was graded as strong (inhibition zone ≥ 70%), moderate (inhibition zone is 50-70%), or weak (inhibition zone < 50%).

$$\text{Antibacterial Activity (\%)} = \frac{A}{B} \text{ x } 100$$

Where: A = clear zone of *D. muscorum* extract (mm) and B = clear zone of antibiotic (mm).

2.6. Microtiter Plate Dillution Assay

Microtiter plate dillution assay was used to know the minimum bactericidal activity (MBC) and minimum inhibitory concentration (MIC) of *D. muscorum* extract against bacterial pathogens that tested positive in the disk diffusion assay (Arguelles, 2022; Arguelles, 2023). Initially, 100 µl of each culture of bacteria were mixed with *D. muscorum* extract (100 µl) at varying dilutions (7.8125 µg/mL – 1000 µg/mL) in a 96- well microtiter plate. The experimental set-up was set aside for 12 hours at 35°C. The MIC of *D. muscorum* extract is the lowest extract concentration that exhibited inhibition of bacterial growth after 12 hours incubation period. MBC of *D. muscorum* extract was assessed by placing a loopful of the test sample (MIC experimental wells that showed no visible growth of bacteria) into newly prepared TSA (tryptic soy agar). The experimental plates were kept for 24 hours at 35°C and were examined for growth of bacteria (colony formation) for each dilution subculturing. Absence of bacterial growth would indicate that the cyanobacterial extract was bactericidal to the test organism at that particular dilution.

2.7. Statistical Analysis

The data obtained from the chemical analyses and antibacterial assays are given as means ± standard deviations of three replicates and was computed using MS Office Excel 2019.

3. RESULTS and DISCUSSION

3.1. Proximate Analysis

Cyanobacteria are important sources of macromolecules like proteins, fiber, carbohydrates, and lipids which are important for food and pharmaceutical applications (Tibbets *et al.*, 2015; Li *et al.*, 2018; Arguelles, 2021; Martinez *et al.*, 2021). The proximate composition of *D. muscorum* is shown in Table 1. Among the macromolecules, carbohydrates and proteins gained the highest concentration with an average value of 35.50 ± 0.12% and 20.19 ± 0.03%, respectively. The observed protein and carbohydrate concentration of *D. muscorum* in this study are within the reported range of *Nostoc* and *Desmonostoc* species from previous studies (Li *et al.*, 2018; Martinez *et al.*, 2021). *Desmonostoc* species are known to produce carbohydrates (heterofucans) such as glucuronic acid and galacturonic acid. The accumulation of carbohydrates in cyanobacteria is a product of the photosynthetic response of these organisms towards nutrient rich conditions wherein carbohydrate content ranges from 10-65% of the total wet weight of the algal biomass (Li *et al.*, 2018; Arguelles, 2021). The protein content of *D.*

muscorum is comparable to several microalgae reported by Tibbets *et al.,* (2015) such as *Nannochloropsis granulata* (17.9%), *Acutodesmus dimorphus* (28.9%), *Porphyridium aerugineum* (31.6%), *Neochloris oleoabundans* (30.1%), and *Phaeodactylum tricornutum* (39.6%). Generally, cyanobacterial species (such as *Desmonostoc* sp.) produce low amounts of lipid since several species of cyanobacteria possess ≤15% lipid of its total dry weight (Tibbets *et al.,* 2015 Li *et al.,* 2018; Martinez *et al.,* 2021). In this study, low lipid content was observed in *D. muscorum* which is similar to that of *N. commune* and *Tetraselmis chuii* with lipid content of 0.26 ± 0.02% and 12.3%, respectively (Tibbets *et al.,* 2015; Martinez *et al.,* 2021). Crude fiber shows the amount of indigestible component (insoluble and soluble fibers) of the algae. On the other hand, ash content reflects the amount of micronutrients present in the sample. The crude fiber and ash content of *D. muscorum* is 7.14 ± 0.13% and 16.90 ± 0.02%, respectively. The amount of crude fiber and ash observed in *D. muscorum* was comparable to those obtained from previously reported species of microalgae such as *Acutodesmus dimorphus, Chroococcus minutus, Chlorella minitussima, Spirulina* sp., and *Botryococcus braunii* (Tibbets *et al.,* 2015; Arguelles, 2021; Arguelles, 2022; Arguelles, 2023). Several factors affect the chemical composition of algal biomass such as growth condition and strain differences. Therefore, differences in proximate composition of *D. muscorum*, in contrast to other microalgal species, were observed in this study. In general, the chemical composition of *D. muscorum* are considered good sources of proteins, minerals, and carbohydrates. The high amount of these macromolecules shows the potential use of this cyanobacterial strain as functional ingredient for food and industrial application.

Table 1. Proximate analysis composition of *Desmonostoc muscorum*.

Proximate composition	Percent composition (%)
Ash Content	16.90 ± 0.02
Moisture Content	5.12 ± 0.01
Carbohydrate	35.50 ± 0.12
Crude Fiber	7.14 ± 0.13
Crude Fat	5.15 ± 0.10
Crude Protein	20.19 ± 0.03

3.2. Elemental Composition Analysis

Cyanobacteria are regarded as rich alternative source of microelements that can be harness for food and agricultural application. These microelements are important for growth and is a product of the overall metabolism of the algal cells (Arguelles, 2018). In this study, ash (minerals and other microelements) generated from complete combustion of algal biomass contains significant amounts of elemental nutrients, such as calcium, magnesium, potassium, and sodium as well as other trace metals present at different concentrations. The mineral composition and their average concentration in *D. muscorum* are shown in Table 2. The mineral distribution present in *D. muscorum* biomass was observed to be in reducing order of Ca > Mn > Mg > K > Na > Fe> Zn > Cr > Pb > Cu > Cd.

Calcium (19245.16 ±7.21ppm) is the most abundant mineral in cyanobacterial biomass, followed by manganese (6372.24 ± 2.45 ppm), magnesium (5733.52 ±117 ppm), and potassium (2723 ± 0.34 ppm). *Desmonostoc muscorum* has higher concentration of these minerals as compared to that reported to *Nostoc commune* with estimated concentration of 21151 ± 833 ppm, 125.98 ± 1.97 ppm, 1959.0 ± 36 ppm, and 1002 ± 2.0 ppm for calcium, manganese, magnesium, and potassium, respectively (Martinez *et al.,* 2021). On the other hand, *D. muscorum* showed comparable concentration of trace elements like zinc (23.78 ± 0.42 ppm), copper (1.27 ± 0.22 ppm), cadmium (0.47 ± 0.32 ppm), lead (1.71 ± 0.64 ppm), chromium (1.94 ± 0.14 ppm), and iron (576.49 ± 0.97 ppm) to that obtained for *N. commune* with estimated

concentration of 22.09±0.27 ppm, 11.88±0.69 ppm, 0.36±0.00 ppm, 3.59±0.05 ppm, 3.77±0.00 ppm, and 4202±37.0 ppm for each trace elements, respectively (Martinez *et al.*, 2021). In the Philippines, limited information is documented on the elemental analysis of microalgal biomass as compared to those reported for macroalgae (seaweeds) (Arguelles & Sapin, 2022). Cyanobacterial biomass generally contains a much lower concentration of inorganic elements (ash) as compared to seaweeds (Tibbets *et al.*, 2015; Martinez *et al.*, 2021). Variation in elemental composition concentration among diverse species of cyanobacteria is common and may be attributed to strain differences as well as culture conditions (like varying light intensity, salinity, and temperature) where the organism was grown (Arguelles, 2022).

Table 2. Concentrations and composition of micro and macro-elements of *Desmonostoc muscorum*.

Cyanobacteria	Elemental Parameter* (in ppm)				
	Ca	Mg	Na	Mn	K
Desmonostoc muscorum	19245.16 ±7.21	5733.52 ±117	1995 ±106	6372.24 ±2.45	2723 ±0.34

Cyanobacteria	Elemental Parameter* (in ppm)					
	Fe	Zn	Cu	Pb	Cr	Cd
Desmonostoc muscorum	576.49 ±0.97	23.78 ±0.42	1.27 ±0.22	1.71 ±0.64	1.94 ±0.14	0.47 ±0.32

* All experimental data are given as mean ± standard deviation (n = 3)

3.3. Antibacterial Activity

Cyanobacteria has been identified to contain promising natural products such as antibiotics and other bioactive substances (Salehghamari & Najafi, 2016; Little *et al.*, 2021). These groups of microorganisms are unexplored and thus serve as a rich pool for the isolation of active compounds (like alkaloids, phenolic compounds, pigments, fatty acids, and terpenoids) that may be of industrial and human importance. *Desmonostoc muscorum* was used to assess its biocidal activity against some medically important bacterial pathogens using the paper disc assay. The occurrence of zones of inhibition was indicative of the culture's ability to produce antibacterial substances. Results of the assay showed that *D. muscorum* possess antibacterial activities by exhibiting zones of inhibition (Table 3 and Figure 2).

Table 3. Antibacterial activities of *Desmonostoc muscorum* extract.

Sample	Antibacterial Activity[a]			
	Gram-positive bacteria			Gram-negative bacteria
	Listeria monocytogenes BIOTECH 1958	*Staphylococcus saprophyticus* BIOTECH 1802	Methicillin-Resistant *Staphylococcus aureus* BIOTECH 10378	*Pseudomonas aeruginosa* BIOTECH 1824
Desmonostoc muscorum BIOTECH 4087	8.57 ± 0.1 (52.58**)	13.7 ± 0.7 (88.20***)	15.7 ± 0.2 (94.05***)	-
Tetracycline[b]	16.3 ± 0.3	15.53 ± 0.3	16.69 ± 0.2	19.7 ± 0.1

Note: [a]Antibacterial activity percentage (%) of algal extracts in contrast to tetracycline: inhibition zone (mm) of algal extract/ inhibition zone (mm) of antibiotic (tetracycline): *** strong inhibition (≥ 70%), **moderate inhibition (50-70%), and *weak inhibition (< 50%) against bacterial pathogen. [b]positive control (antibiotic). (-) no zone of inhibition.

The cyanobacteria exhibited inhibition zones of 13.7 ± 0.7 mm, 8.57 ± 0.1 mm, and 15.7 ± 0.2 mm against *Staphylococcus saprophyticus, Listeria monocytogenes,* and Methicillin-Resistant *Staphylococcus aureus* (MRSA), respectively. The algal extract showed strong inhibition against medically important *Staphylococcus* species (*S. saprophyticus* and MRSA) and moderate inhibition against *L. monocytogenes*. On the other hand, *D. muscorum* extract showed

no antibacterial activity against *Pseudomonas aeruginosa*. The zones of inhibition of *D. muscorum* extract against Methicillin-Resistant *Staphylococcus aureus* (MRSA) is more potent than that obtained for *N. commune* (zone of inhibition = 0.9 mm), *Nostoc linkia* (zone of inhibition = 0.9 mm), and *Acutodesmus dimorphus* (zone of inhibition = 15.1 ± 0.3 mm) but is less effective to other algae such as *Oscillatoria princeps* (zone of inhibition = 18.8 mm) (Shaieb *et al.*, 2014; Yalcin *et al.*, 2022; Arguelles 2023).

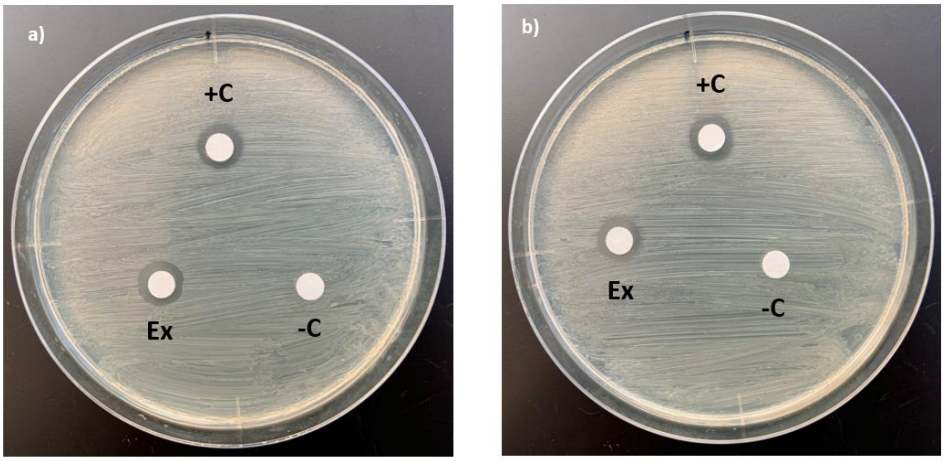

Figure 2. Inhibition zones exhibited by *Desmonostoc muscorum* methanol extract against (a) Methicillin-Resistant *Staphylococcus aureus* and (b) *Staphylococcus saprophyticus.* (-C) methanol/negative control, (+C) tetracycline/positive control, (Ex) extract.

The findings of the paper disk assay for *D. muscorum* extract were further validated by determining the MIC and MBC values of the cyanobacterial extract using microtiter plate dilution assay (Table 4). Results of the assay showed potent antibacterial activities against *S. saprophyticus* and Methicillin-Resistant *S. aureus* (MRSA) both with MIC and MBC values of 125 µg/mL and 250 µg/mL, respectively. Additionally, MIC (250 µg/mL) and MBC (500 µg/mL) values against *L. monocytogenes* showed less potent activity as compared to other tested bacterial pathogens. The antagonistic activity of *D. muscorum* extract against *L. monocytogenes* is more potent as compared to *Moorea producens* which exhibited MIC value of >500 µg/mL (Dussault *et al.*, 2016). In addition, *D. muscorum* extract showed similar antibacterial activity against *S. saprophyticus* (MIC = 125 µg/mL and MBC = 250 µg/mL) to that of *Nostoc commune* (Martinez *et al.*, 2021). However, *N. commune* did not show biocidal activity against *L. monocytegenes* whereas *D. muscorum* exhibited potent antibacterial activity. El-Sheekh, *et al.*, (2006) revealed that *D. muscorum* was able to exhibit zones of inhibition against a broad spectrum of bacteria like *S. aureus*, *Escherichia coli*, *Salmonella typhi*, and *Bacillus cereus*. These activities are said to be caused by substances such as phenolic compounds, polyketides, amides, fatty acids (palmitoleic and linoleic acids), terpenes (β-ionone, neophytadiene), alkaloids and peptides that are naturally present in the organism.

Table 4. MIC and MBC of *Desmonostoc muscorum* extract.

Bacterial Pathogens	MIC values (µg/mL)	MBC values (µg/mL)
Listeria monocytogenes BIOTECH 1958	250	500
Staphylococcus saprophyticus BIOTECH 1802	125	250
Methicillin-Resistant *Staphylococcus aureus* BIOTECH 10378	125	250
Pseudomonas aeruginosa BIOTECH 1824	>1000.00	ND

*ND = None Detected; MIC= Minimum Inhibitory Concentration; MBC= Minimum Bactericidal Concentration

Desmonostoc muscorum extract is considered more effective in inhibiting Gram-positive bacterial pathogens as compared to *Pseudomonas aeruginosa*. Gram-negative bacterial species are characterized to have thin cell wall (with peptidoglycan) and outer membrane (with specialized proteins and lipopolysaccharides). These cellular structures act as protective barrier against potent antibiotics making Gram-negative bacteria more stable than Gram-positive bacterial strain (Preisitsch *et al.*, 2015). In addition, the production of antimicrobial agents in cyanobacterial extract is also influenced by important factors during cultivation condition such as components of the growth medium, light intensity, temperature, and pH (Orhan *et al.*, 2003; Katircioglu *et al.*, 2005). Thus, it is possible that varying growth conditions of *D. muscorum* can lead to different patterns of antibacterial activities which can be a subject of future studies using this cyanobacterium. The study documented the potent antibacterial activities of *D. muscurom* against clinically important bacterial pathogens. To date, this study documents for the first time the antibacterial activities of *D. muscorum* extract against *L. monocytogenes* and Methicillin-resistant *S. aureus* (MRSA). The methanol extract of *D. muscurom* contains bioactive compounds causing these biological activities. Thus, additional studies that will target the isolation and identification of these compounds is recommended to further elucidate the biocidal activity of the extract.

4. CONCLUSION

In conclusion, the study documented that *Desmonostoc muscorum* possesses high concentration of important biomolecules such as carbohydrates, proteins, and microelements. In addition, *D. muscorum* can be utilize as potent source of antibiotics against clinically important bacterial pathogens. It is recommended that further experimental investigations should be done focusing on isolating and identifying the bioactive compounds in *D. muscorum* extract. Additionally, performing *in vivo* toxicity assays is important to substantiate the safety and efficacy of the *D. muscorum* extract for potential medical use. Further studies on the antibacterial diversity of this cyanobacterium are also recommended to assess the practicality of scaling up the production of *D. muscorum* for applications in the food and pharmaceutical industries.

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Declaration of Conflicting Interests and Ethics

The author declares no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author.

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